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Histomorphological Study of the Olfactory Bulb in Local Breed Sheep (*Oves aris*)

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The objective of the current study was to assess the morphological and histological characteristics of the sheep olfactory bulb, six samples (n = 6) were used. Three samples for anatomical observation of olfactory bulb were record in adult sheep which involved the morphological study (stance, rapport, mass, length, and diameter). The width of the bulb was (7.28± 0.98mm), the length of the olfactory bulb in sheep was (30.5±1.28 mm), the diameters of the olfactory was (9.067-+0.01453). The olfactory bulbs were fixed in 10% formalin solution and subjected to standard histological processing, which included Mallory and Hematoxylin and Eosin staining. The olfactory bulb, located in the forebrain of vertebrates, has an elongated form with just a rostral ventral rough surface and a creamy color. It receives neurological input regarding odors identified by cells in the nasal cavity. The four separate layers that may be distinguished histologically in the sheep olfactory bulb are the olfactory nerve layer, the glomerular layer, the mitral cells layer, and the granule cells layer.

Keywords: Olfactory bulb, sheep, glomerular layer, mitral cells layer, Formalin fixation, Mallory staining, Hematoxylin and Eosin staining, Forebrain, Olfactory nerve layer, Glomerular layer, Mitral cells layer.

INTRODUCTION

One of the conventional five sensory faculties, smell, plays a crucial role in how animals behave and interact with their environment. In small ruminant, feeding and foraging, inter- and intraspecific communication, environmental awareness, predator avoidance, mating and reproduction, and territorial marking are just a few of the many tasks it performs (Mota-Rojas, *et al*., 2018). However, the mother (ewe) performance in the first birth is representative of her performance in the next births. The sensory quality of each ewes affects maternal behavior, and calm sheep seem to be better mothers than nervous sheep (Lévy, 2022).
So, different senses are involved in the formation of maternal behavior and its continuation for lamb caring (Dwyer and Lawrence, 2000). The sense of smell seems to play the most important role in this, and sheep will not allow them to breastfeed until they are sure of the smell of their lamb (Le Neindre and Poindron, 1990).
But, some studies have shown that the lack of olfactory power does not interfere with the formation of maternal behavior, and that sheep tries to compensate for the lack of olfactory power with the help of other senses (Ferreira *et al*., 2000; Nowak and Boivin, 2015).

The highly organized olfactory bulb, which is located in the forebrain of vertebrates, receives neural input about odors that are detected by cells in the nasal cavity through direct extension of olfactory receptor (smell receptor) cells' axons into the structure (Reshag, 2023). The olfactory bulb is a main component of the olfactory system in animals in which it plays an important role as the interface between the peripheral components and the cerebral cortex responsible for olfactory interpretation and discrimination (Lledo *et al.*, 2005).
Olfaction is the ability to recognize odorous chemicals that have been aerosolized in the environment. Olfaction is one of the senses, along with vision, taste, hearing, and balance Reshag (2023). According to Scott *et al.* (1993), the olfactory system's components enable humans to sense odors. In summary, the primary or auxiliary olfactory system is used for odor reception and processing.
The olfactory sense is exceptional, special, and important for overall animal consciousness. According to (Reed, 1999; Padodara and Jacob 2014), it is utilized for finding, recognizing and differentiating between foods, communicating, interacting, navigating, recognizing, predator avoidance, mating, and territoriality. Based on these background and importance of these organs, the aim of present study was to assess the morphological and histological characteristics of the local sheep's olfactory bulb.

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MATERIALS AND METHODS

***Animal's collection*:** For this investigation, six olfactory bulb samples from healthy adult sheep (1-3 years) were used. After the animals were killed, the heads were removed from the bodies, and the brains were examined to identify where and how the olfactory bulbs were connected. After that, the bulb was removed in order to measure the morphological traits, including length, width by vernal caliber, and weight by digital electronic balance, and color (Naser and Khaleel, 2020). For histochemical analysis, the olfactory bulb samples were immediately fixed with neutral buffer formalin 10% and rinsed with normal slain (Alfahdawi and Ottman Alfahdawi, 2023).

***Samples preparation*:** The specimens were prepared using the paraffin embedding method (Zebon *et al.*, 2020), According to Al-Taai and Hussin (2015), the structure was separated into three sections: anterior, middle, and posterior. The tissues were sectioned at a thickness of 5 m using a rotatory microtome, and the tissue slices were stained with hematoxylin and eosin and Mallory stain to show the kind of connective tissue.

The stereoscope was used to evaluate the morphological images while the Future Win Joe China 5 Megapixel digital camera was used to record the histology photographs. The Statistical Package for the Social Sciences (SPSS) version 13.00 (Joda, 2008)'s t-test was used to statistically analyze the experimental results (Khaleel *et al*, 2017).

To analyze the numerical data gathered from sheep, the Statistical Package for the Social Sciences (SPSS) application (version 16.0; Chicago, USA) was utilized, and we used the Student's t-test. Data were presented as means with standard errors (SE), correlation coefficients, and statistical significance at the 95% level of confidence (p 0.05) with n=6 for each (Khaleel *et al*, 2017)

RESULTS AND DISCUSSION

***Morphologically stud*:** The results showed olfactory bulb, it was located in the fore brain. sheep olfactory bulb had elongated in shaped, creamy in color (figure 1) and vertebrate forebrains contain a component that receives neural information about odors picked up by cells in the nasal cavity. The frontal lobe's orbital aspect is just below the paired olfactory bulb, which is located in the anterior cranial fossa. It is located anterior to the cribriform plate of the ethmoid bone and laterally to the crista gale. The olfactory bulb is located on a flat band of nerve fibers called the olfactory tract at the anterior extremity of each hemisphere. Two diverging bands known as the medial and lateral olfactory striae (figure 2) continue this tract behind. The olfactory stria, which extends posteriorly as the olfactory tract, is located above the olfactory bulb (figure 3).

The width of the olfactory bulb in sheep was (7.28± 0.98mm), but the length of the olfactory bulb was (30.5±1.28 mm), the diameters of the olfactory was (9.067-+0.01453), and the weight of the olfactory bulb was (0.8567-+0.07446).

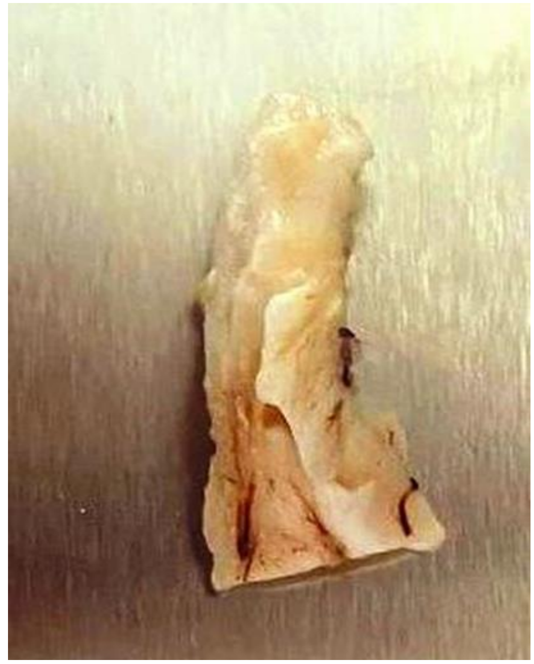

Figure 1. Photograph of the gross anatomy of the olfactory bulb of sheep shows: olfactory bulb (O) Elongated shape, creamy in color and large, and olfactory tract (T).

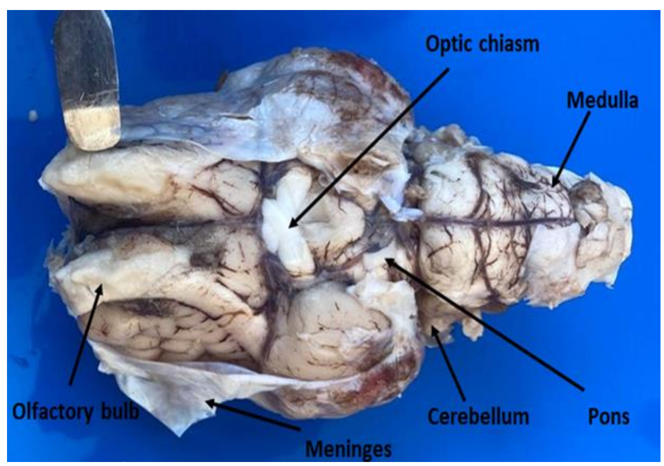

Figure 2. Photograph of the topography and relationship of the olfactory bulb in sheep brain shows: (O Optic chiasm, M Medulla, OL Olfactory bulb, Me Meninges, P Pons, Ce Cerebellum). (The olfactory tract is situated on a flat band of nerve fibers at the front end of each hemisphere of the brain).

Figure 3. The olfactory bulb, the olfactory tract, and the olfactory stria are seen in a photograph of the brain's inferior view. The olfactory bulb is located above the olfactory tract, which continues as the olfactory stria posteriorly.

Histological features of the MOBs: The Morphology of olfactory nerve was explained previously, but the histologically in (figure.4A, B), for the determining of MOBs sections were stained with H&E, different layers of MOBs in sheep. The olfactory nerve was organized histologically in multi layers from outsides toward insides in all species as a, the periventricular layer (PVL), the granule cell layer (GCL), the internal plexiform layer (IPL), mitral cell layer (MCL), glomerular layer (GL), exterior plexiform layer (EPL), and external plexiform layer (EPL). IPL is not noticeable in sheep. Additionally, the olfactory ventricle's lining, known as the ependymal layer (EL), was initially identified in sheep. The outermost layer was composed of the ONL, which primarily produced the olfactory nerve axons that were unsheathed by olfactory unsheathing glial cells (OECs) with oval cell bodies and pale cytoplasm. Anyway, few numbers of olfactory axons in the sheep were enclosed the few processes of OECs. The olfactory nerve fascicle was formed when these olfactory axons congregated. The GL, which included what are known as glomeruli, was located just beneath the ONL. They circled the olfactory bulb's exterior completely. The glomeruli in sheep were often placed in a double layer and were small (285-395 μm in diameter). Between the glomerular layer and the mitral cell layer, the external plexiform layer, which was the second-largest layer, was present. One of the cells that are evenly dispersed over the entire layer of the EPL is the tufted cells. Additionally, the dendrites of tufted cells, mitral cells, and granule cells formed the neuropil of the EPL (figure 4D). There are also a lot of juxtaglomerular cells, which penetrated the glomerular neuropil in close connection. These cells were primarily seen on the ventral and lateral sides of the sheep's glomeruli, as well.

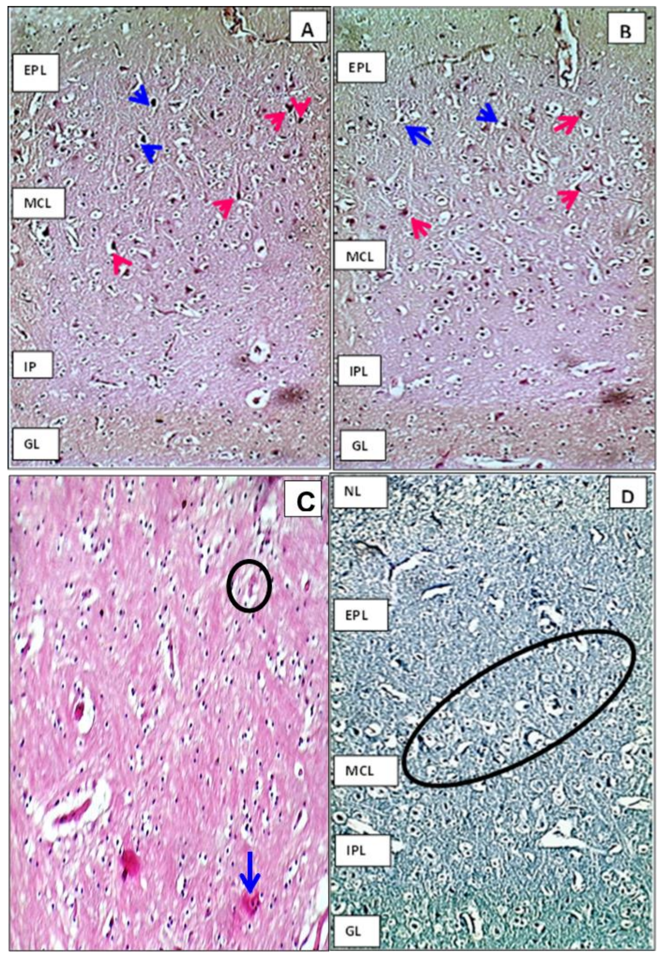

Figure 4. Histological study of the mitral cell layer of the olfactory bulb of sheep, of the mitral cell layer (MCL) showing typical (red arrows) H&E, (black circle) Mallory stain and deep tufted cells (deep blue arrows) scattered along the external (EPL) and internal plexiform layer (IPL), in addition the granular layer (GL), H & E stain x 100. C) Showing scattered neurons with large somas are also found (black circle) and neuropil (asterisks) (blue arrow) H & E stain x 100 (A,B,D).

The other layer, which has a single row of mitral cells and many interneurons with spherical cell bodies, is thought to be the thinnest layer (figure.4,C). The MCL in the sheep were tiny, elongated, and had oval nuclei in the center. After the mitral cells layer, the internal plexiform layer (EPL) was the fifth layer and was much thinner than the EPL. In sheep, this layer had neuropil (mitral cell axons and granule cell dendrites), but the granule cell bodies were rarely distinguishable. The last, largest, and deepest layer of the MOB was composed of granular cells. A vast number of granule cells that range in shape from round to ovoid define this layer in sheep. The GCL is divided into inner and outer sub-layers as a result of the density and placement of these layers. The cells were grouped in clusters of several, then of a few, cells in the inner sublayers. Because the cells in the outer sublayers are firmly packed together, other species, such as carnivores, have longer fusiform bands than sheep. The spaces between these cell bands are also wider. One of the several layers that were observed in the olfactory bulb is the PVL (sub-ependymal layer), which encircles the whole circuit of the olfactory ventricle. The layer contained many tangentially oriented axons and a small number of cell bodies that were haphazardly organized. However, the EL was seen as the last, thin layer of the MOB and bordered the olfactory ventricle lumen. There were few cell bodies and a very high density of nerve fibers in this layer.

DISCUSSION

MOB locations in the current investigation varied by species. In the ventral and lateral images of the dog's and goat's brains, respectively, MOBs were clearly visible.

In a previous study by Ibe et al. (2006), the elephant olfactory bulbs were hidden beneath the frontal lobes, while the adult African grass cutter olfactory bulbs were apparent on the dorsal brain image in this examination.

The basic histological organization of the MOB in rodents was described by Wei et al. (2008), as having six layers in the study of sheep. The sheep had seven layers: ONL, GL, EPL, MCL, IPL, GCL, and PVL stayed constant throughout. Studies claim that the African elephant had the similar result Ngwenya *et al.* (2011), According to various research on elephants Ngwenya *et al.* (2011); Marschner. (1970) and a prior study on goats Tillet et al. (1987), the internal plexiform layer of the studied sheep was indistinct. In contrast to carnivores, sheep had both PVL and EL, but carnivores only had PVL, which was the parent of numerous olfactory bulb cells (Shipley and Ennis,1996).

Due to the synaptic interaction that occurs there between mitral cells and olfactory receptor axons, the granular layer is thought to constitute the functional unit of the Kosaka (2005), Additionally, the GL revealed species differences in mammals, particularly in the complexity of the olfactory glomeruli in animals with highly developed olfactory abilities, as detailed in research on the dog and the fox. In contrast, species with less developed olfactory receptors, as domestic chickens (Gallus gallus), displayed typical glomerular shape. Lee *et al.* (2019) findings were examples of individuals who fit this description.

5-Comparing the rat and pigeon olfactory bulbs for parvalbumin-immunoreactive cells. Embryol Histol Anat.

6-Our research made it clear that the glomular layer was made up of per glomerular and olfactory glomeruli. In contrast to the other animals in the dog, which were larger and placed in one layer, sheep's olfactory glomeruli were small in size and arranged in double layer. This trait is common in many mammals, but the African elephant exhibits up to four levels of it Manger. (2011), Additionally, we noticed that sheep had fewer juxtaglomerular and glia cells than other mammals, contrary to what has been described for birds and fish. It had a poor sense of smell (Steiger *et al.* 2009). Greer *et al.* (2008) discovered that olfactory glomeruli are where odor signals are processed before being sent to the EPL along the major dendrite of the mitral.

7-Most of the tufted cells and mitral and granule cell dendrites that are dispersed throughout the sheep's exterior plexiform are granule and mitral cells. Compared to other animals like the dog, this layer is thinner in sheep. These may be to blame for the increased odor propagation along the primary dendrite of mitral cells, according to Mori *et al*. (1983), and Lahr *et al.* (1990), In a sheep study, the MCL was composed of major neurons, one of the MOBs, and their enormous somata. Mitral cells in sheep are small, elongated, and have an oval nucleus in the center. Numerous studies such as Nagayama et al. (2014) and Ngwenya *et al.* (2011), have shown that the morphology of mitral cell somata varies based on the species of animal. This variation may be the cause of the high odor sensitivity in various animals because of the significance of mitral cells in olfactory signal transduction to higher brain areas (Wei *et al.*, 2008)

According to Kosaka (2009), the granular cells layer is the MOB's thickest layer. When compared to sheep, GCL in the examined carnivores, such as the dog and fox, was significantly larger and featured clusters of densely packed granule cell somata. Gap junctions between them allow them to synchronize their functions (Shipley and Ennis, 1996), Our most important observations about the ONL relate to the olfactory nerve axons and OECs, particularly OECs processes, which are not equally distributed in the ONL in sheep.

***Conclusions*:** The basis of this work, we conclude the following:

1. The fundamental histological structures of the MOB in rodents were characterized in six layers in the research of sheep. The sheep had seven layers: ONL, GL, EPL, MCL, IPL, GCL, and PVL stayed constant throughout.
2. Olfactory abilities with less in sheep.

3. In contrast to carnivores, which had just PVL, which was the parent of numerous olfactory bulb cells, sheep had both PVL and EL.
4. The sheep's olfactory glomeruli were tiny and organized in a double layer.
5. The juxtaglomerular and glia cells few in sheep.

***Authors' contributions*:** Qasem sami jawad and Khayreia K. Habeeb, designed and completed the experiments, Qasem sami jawad finalized the draft.

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Ethical statement: This experiment has been designed based on regulation of ethical committee of veterinary faculty of Baghdad university.

***Availability of data and material*:** completive data can be obtained when requested.

***Consent to participate*:** All authors participated in this research study.

***Consent for publication*:** All authors submitted consent to publish this research article in JGIAS.

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